Epigenetic Aging Clocks:

Measuring Mortality

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SUMMARY

This term paper is a short review of the capabilities and history of epigenetic aging clocks, focusing on possible uses in both nonhuman and human longevity research. I will begin by covering the conceptual background required for understanding the function and purpose of DNA methylation-based aging clocks, first reviewing various prior methods of measuring aging before defining epigenetic age and explaining the basic principles behind epigenetic aging clocks. Then, I will cover the history of epigenetic aging clocks and their transition from chronological age indicators into mortality predictors, before focusing on their current uses in both human and nonhuman animals. I will conclude the paper with an analysis of underutilized opportunities for the use of these clocks in biodemography and chronic disease screening.

INTRODUCTION

Over the last several decades, the field of epigenetics has moved from a relatively niche study of gene expression to the forefront of aging research. Epigenetic aging clocks have been critical in demarcating the difference between chronological and biological age, and in enabling a more direct study of the latter. These clocks work by observing the DNA methylation (DNAm) patterns across a variety of CpG sites (sites where a cytosine is followed by a guanine on the genome, where methylation can take place) of interest which are strongly correlated with aging and aggregating this data to predict either the chronological or biological age of an individual, depending on the clock design. Both the number of CpG sites surveyed and the accuracy of the predictions of the DNAm clocks have increased over time, and they have already been used as indicators for both the mortality risk of an individual and the success of anti-aging interventions (Fahy et al., 2019). The potential DNAm clocks have to be used as biodemographic aides and as screening tools in humans, however, has thus far been mostly neglected. More epigenetic aging clocks should be devised for model organisms in the near future, and the implementation of epigenetic assays as screening tools for age-related diseases should be more widely considered.

BACKGROUND

Measuring Aging

The age of humans has traditionally been measured simply by counting the number of years a person has been alive since birth, also known as their chronological age. There is no doubt that chronological age is intrinsically tied to the rate of actuarial aging, which can be modeled impressively well by the Gompertz model, designed in 1815. The Gompertz model suggests that the risk of death in humans increases by approximately 8% each year after 30 until around 80 years old, and this theory matches observed demographic data well. (Gompertz, 1815) However, individual humans vary greatly in their level of frailty and risk of death, which causes most of the observed difference in human actuarial aging. (Hawkes et al., 2012) Thus, while chronological age is a good predictor of the biological age of an organism, it is not ideal for predicting individual outcomes.

Additionally, the simple passage of time is not enough to explain the aging process, as individuals exposed to stressors like famine, droughts, war, and poverty, or environmental toxins often display phenotypical signs of aging earlier, as do people suffering from alcohol (Leber, 1982) or tobacco (Morita, 2007) addictions. The Gompertz

model is thus of course outperformed in predicting the risk of death of any given individual by models that take environmental and historical data into account (Jylhävä et al., 2017). Even still, gathering data about individual risk exposures is at best difficult and time-consuming, and often impossible in many locations. Thus, it is necessary to discover effective biomarkers of aging, which can easily and replicably be observed without harming subjects. Most molecular biomarkers of aging that reflect environmental risk factors do not meet these criteria. However, because gene expression in humans is significantly altered by the environment, novel methods of measuring aging that study the epigenome, which is the sum of nonstructural changes to the genome that adjust gene expression in cell lines and their descendants (Bernstein et al., 2007) have been devised. The most relevant epigenetic alteration for measuring biological age is direct methylation of DNA, as other regulatory factors like acetylation of histones are much more transient in nature, with most failing to persist through the DNA replication process (de la Parte, C, & Guallar, D., 2023).

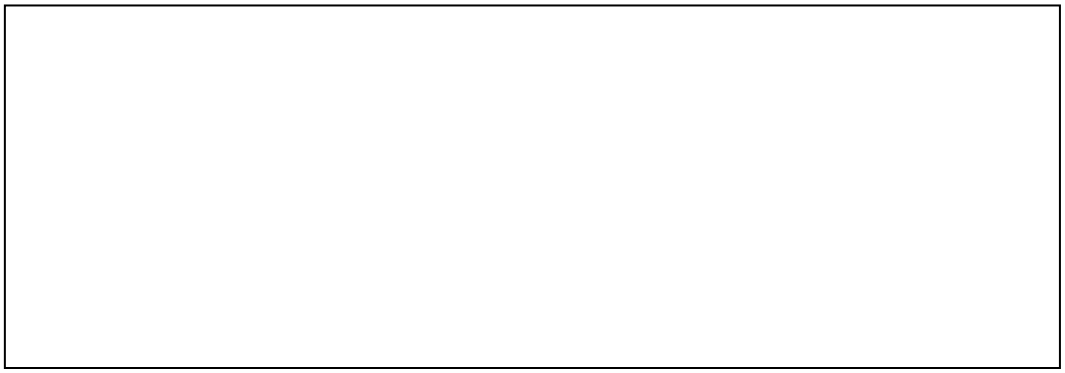
Methylation and CpG Sites

DNA methylation is chemically trivial in animals: at each CpG dinucleotide, which is simply a cytosine nucleotide followed by a guanine nucleotide (Li et al., 2022), a methyl group may be added to the genome by one of many proteins. The process of methylation is common to essentially all life, and it is one of many epigenetic methods that allow organisms to regulate the transcription of genes without altering their genetic code (Bernstein et al., 2007). Methylation at CpG sites in the genome force that part of DNA to coil more tightly around its histone, which makes it difficult for proteins to access it. This is likely why CpG sites are so common in the promoter region of genes: they allow for easy regulation of the transcription of a gene simply by preventing RNA transcriptases from ever being able to form a complex and begin transcription. DNA methylation is also heritable across instances of mitosis (Bernstein et al., 2007), which means that all daughter cells of a given cell will carry nearly the same epigenome as their progenitor. It is also somewhat heritable from mother to daughter across meiosis, though much of the epigenome is reset during that process. Not all CpG sites are correlated with aging or environmental stressors, but the methylation status of some CpG sites is highly correlated with increased age. Thus, identifying a large number of sites correlated with age and assaying their methylation status can provide a good estimate of the age of an individual, both chronologically and biologically. This is the main objective of DNAm aging clocks.

History

The history of epigenetic aging clocks begins in 2011 with the creation of the first epigenetic predictor of age (Bocklandt et al., 2011). Bocklandt and colleagues analyzed thousands of CpG sites in twins ranging from 18 to 70 years of age, looking for locations where methylation status was heavily correlated with chronological age. While the Bocklandt predictor made no attempt to estimate biological age, it was able to estimate chronological age with an average accuracy of 5.2 years simply by observing the methylation patterns across three CpG sites. Horvath was the first to create an epigenetic aging clock with the express goal of designing a multi-tissue predictor of age, which tracked 353 clock CpG sites (Horvath, 2013) with an average accuracy of 3.6 years, as shown in Figure 1. The clock was capable of predicting the age of chimpanzees with some accuracy, but it was less useful for other primates like gorillas.

In comparison, Porter et al.'s modern chronological DNAm clock, created in the same way as Horvath's but with many more sites



included (Porter et al., 2021), had an average accuracy of 3.7 years, which indicates that there are diminishing returns for including ever more CpG sites in DNAm clocks. Tissue-specific clocks like the blood-based clock created by Zhang et al with a best-performing average error of 2.04 years (Zhang et al., 2019) can be better predictors of chronological age than pan-tissue clocks like the Horvath and Porter clocks, but they can be greatly skewed by tissue-specific diseases. This also raised the question: should epigenetic aging clocks still be attempting to predict chronological age as a proxy for biological age, or would they be more useful attempting to predict age-related phenomena like mortality rates or chronic disease incidence?

CONTEMPORARY USES

Mortality Predictors

Epigenetic aging clocks diversified rapidly over the course of the 2010s, as several teams analyzed further CpG sites and began to devise new types of tissue-specific and pan-tissue DNAm clocks. One significant milestone in the development of epigenetic aging clocks was the observation that epigenetic age could act as a better predictor of all-cause mortality than chronological age. (Chen et al., 2016) People that had a higher predicted "epigenetic age" based on the Horvath and Hannum clocks than their chronological age were much more likely to die sooner than their non-age-accelerated counterparts, with the 5% fastest epigenetic agers having a 48% higher risk of

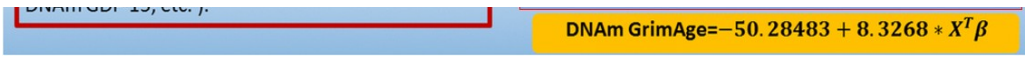


Figure 2: Methods used to create the DNAm GrimAge mortality predictor (Lu et al., 2019).

death. After this milestone was reached, various competing clock teams began focusing on building better predictors of mortality. Chronological clocks, which simply attempted to estimate the time an individual has been alive, were deprioritized relative to biological clocks (Li et al. 2022), which, like their predecessors, have also begun to diversify. The first DNAm clock designed to track biological age over chronological age was dubbed DNAm PhenoAge (Levine et al., 2018), which was created in 2018 and was the first epigenetic aging clock specifically designed to incorporate healthspan. In 2021, the leading

epigenetic clock for the prediction of mortality was the aptly named GrimAge clock (McCrory et al., 2021). The GrimAge clock (Lu et al., 2019) fully moves away from predicting chronological age, instead consisting of a DNAm surrogate for smoking pack-years and 11 DNAm surrogates for blood plasma protein levels, as shown in Figure 2, much of which is taken directly from Lu et al.'s methods.

The smoking pack-year indicator is still capable of predicting time-to-death in people who have never smoked, and is a better indicator for the lifespan of smokers than their self-reported number of packs smoked. Additionally, GrimAge is strongly predictive of the onset of major mortality contributors like coronary heart disease and cancer. It is likely that still more accurate mortality predictors will be produced by folding more DNAm surrogates for other biomarkers of aging into a single model, but GrimAge is currently the clock of choice for studying longevity interventions and mortality.

Trials and Interventions

DNAm clocks have already seen some use in the field of longevity interventions in humans. Perhaps more importantly than their ability to act as an indicator of the success of aging interventions, DNAm clocks can also tell longevity researchers quickly if their interventions are unsuccessful, thereby preventing funds and time from being wasted on studies that would otherwise consume a great deal of time and money. However, there have been some notable successes in the field measured using epigenetic aging clocks. For example, the DNAm GrimAge clock was used to measure the success of a thymus regeneration protocol in 2019, which was the first recorded "increase, based on an epigenetic age estimator, in predicted human lifespan by means of a currently accessible aging intervention." (Fahy et al., 2019). DNAm clocks can also be used in vitro to study the effects of reprogramming factors on human cells, which has been the method most capable of reversing epigenetic age in the laboratory (Gill et al., 2022). Gill et al. were able to achieve an estimated decrease in epigenetic age of 30 years in vitro without the loss of original cell identity through maturation phase transient reprogramming, which is highly significant, given the low mean error rates of DNAm clocks.

Novel uses

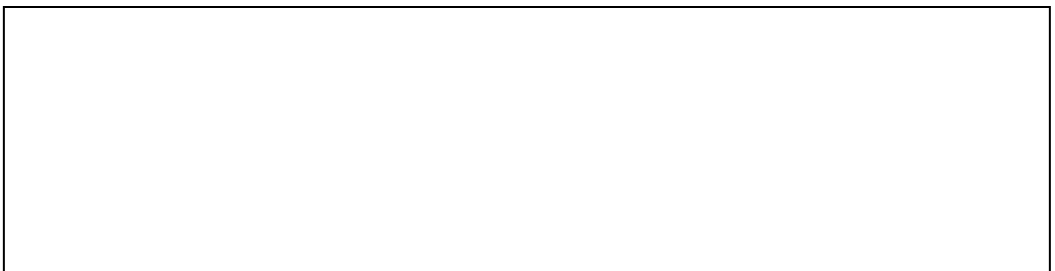
Environmental Analysis & Screening

One of the biggest problems in human demography is the difficulty demographers have in estimating the frailty of individuals. Population frailty can be estimated by death rates over time, and environmental risks identified, but it is challenging to provide

now providing evidence for epigenetic predictors of the development of type 1 diabetes (Johnson et al., 2020). One of Johnson et al.'s figures is included above, indicating that hypermethylation of the LHX6 gene is linked to the development of type 1 diabetes, as are several other epigenetic changes. Screening for these epigenetic changes in individuals who are already genetically prone to T1D, could eventually allow early intervention and treatment in order to prevent the cardiovascular damage that usually occurs with the disease (DiMeglio et al., 2018), or even enable study of its pathogenesis, which is still not well understood. As of right now, such research is currently ongoing in several labs (Crna, 2019), (Zhang et al, 2021). Because DNAm aging clocks and mortality predictors are excellent predictors of many different chronic diseases, epigenetic screening could allow us to screen for many more diseases beyond type 1 diabetes. It could also allow us to begin screening for environmental pollutants at the same time as other diseases, saving a great deal of time and money, if we were able to discover sites whose methylation was correlated with exposure to such pollutants.

Biodemography

Unlike most methods of studying the biologically damaging effects of age in various organs, DNAm aging clocks do not require particularly intrusive tissue sampling (Jylhävä et al., 2017), and were thus more apt for immediate human study. This is likely responsible for the significant discrepancy in use between nonhuman epigenetic clocks and human clocks. Epigenetic aging clocks have been used in some model animals, like mice and chimpanzees (Horvath, 2013), but they have not yet been applied in many studies. It was 6 years after the first epigenetic clock's creation that a similar clock was made for mice (Wagner, 2017), and three years later, in 2020, that the first clock was designed for zebrafish (Mayne et al., 2020). Zebrafish were an excellent choice for designing a clock, since their methylome is well



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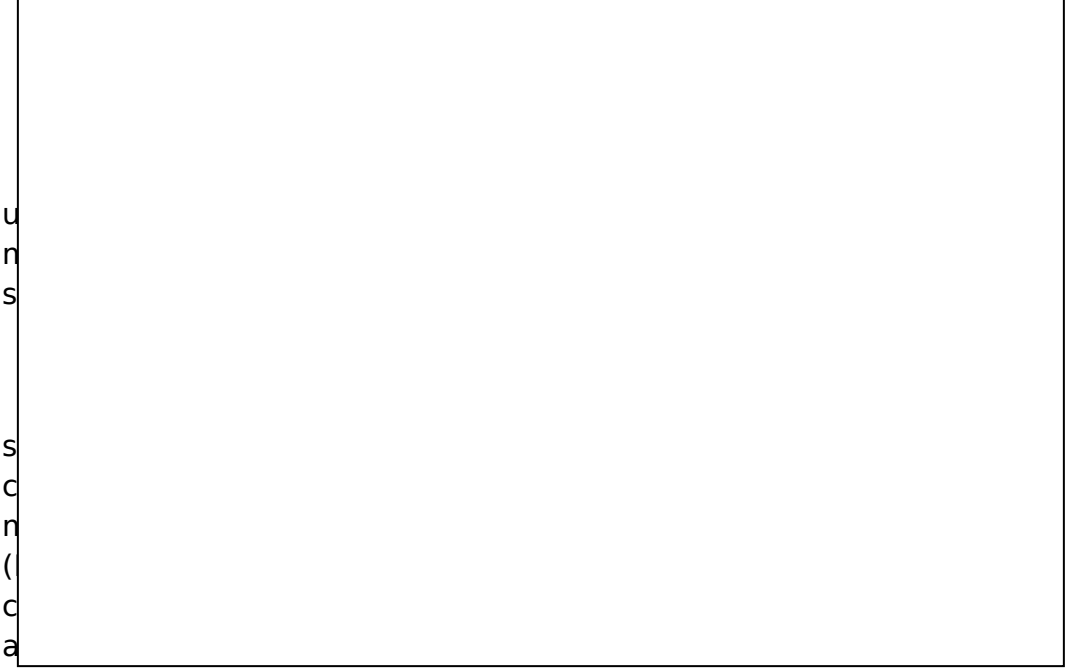

Horvath's lab was able to create an epigenetic clock for the naked mole rat, a notoriously long-lived eusocial mammal, which showed significant differences in epigenetic age between queen and non-queen rats. This preliminary research may lead to further understanding of the epigenetic components of their extraordinary longevity. Beyond enabling less invasive and rapid study of vertebrates, DNAm clocks can also help solve a common problem faced in biodemography: the question of where to begin tracking the life of an organism. Conception and birth are common choices, as are the beginnings of adult life in animals with larval stages. Epigenetic aging clocks can provide a method of studying aging in early development post-conception, accurately predicting age "back to 8 weeks of gestational age, and likely to conception" (Hoshino et al, 2019).

Conclusions

In conclusion, DNAm-based aging clocks have rapidly moved from predicting chronological age to predicting biological age and risk of death over the course of the last decade, and they are an excellent tool for predicting both human mortality and the effectiveness of longevity interventions. However, they are significantly underutilized in nonhuman animals, with the possible exception of mice, which could be detrimental to our understanding of the underlying processes behind aging. Early studies of many important model vertebrates for biodemography via DNAm clocks have only started in the last three years, and so this space is likely to grow rapidly in the coming years. DNAm predictors could also be used to screen for the early onset of chronic diseases in humans and aid in the study of the etiology of these diseases, acting before traditional screening methods.

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